Microhabitat preferences of the endangered Growling Grass Frog[*] *Litoria raniformis* in southern Victoria

Geoffrey W. Heard[1], Peter Robertson[2] and Michael P. Scroggie[3]

[1]Department of Zoology, La Trobe University, Bundoora, Victoria, Australia 3086.
Email: gwheard@students.latrobe.edu.au

[2]Wildlife Profiles Pty. Ltd., PO Box 572, Hurstbridge, Victoria, Australia 3099.
Email: wildlife.profiles@bigpond.com

[3]Arthur Rylah Institute for Environmental Research, P.O. Box 137, Heidelberg, Victoria, Australia 3084.
Email: michael.scroggie@dse.vic.gov.au

ABSTRACT

We examined nocturnal microhabitat preferences of the endangered Growling Grass Frog *Litoria raniformis* in lotic and lentic environments in southern Victoria, Australia. Data were obtained during surveys of six wetland sites during the summer of 2003. At all sites the observed distribution of frogs amongst microhabitat categories differed from their availability, as assessed by sampling of random points. Frogs were located most often on bare soil, bare rock or leaf litter when on land, and on floating, submergent and emergent vegetation in aquatic situations. Non-metric multidimensional scaling and analysis of similarities (ANOSIM) were used to compare the structural attributes of microhabitats used by *L. raniformis* to those of random points. In both the riparian and aquatic zones of the study sites, microhabitats used by these frogs differed from random points in their degree of vertical structural complexity. Whilst our data may be biased by the observability of frogs in different microhabitats, this study suggests that structurally open microhabitats are an important component of the habitat of *L. raniformis*. The ecological basis for this result is discussed, as are implications for our understanding of the species' habitat requirements.

Key words: *Litoria raniformis,* endangered, microhabitat preferences, habitat requirements, conservation, Victoria.

Introduction

For most threatened amphibians, elucidating their habitat requirements remains a fundamental step for conservation planning. There are three main reasons for this. Firstly, amphibians may be sensitive to even minor habitat alterations, due to their complex life-cycles and physiological dependence on the immediate environment (Cushman 2006). Secondly, only basic information is available on the habitat requirements of most taxa (Hazell 2003; Cushman 2006). Thirdly, habitat alteration is considered the principal process endangering amphibians globally (Stuart *et al.* 2004).

To gain a preliminary understanding of the habitat requirements of wildlife, and to provide guidance for research and conservation planning, conservation biologists often employ statistical techniques to explore relationships between spatial variation in habitat attributes and habitat occupancy (Burgman and Lindenmayer 1998). These relationships are of interest because habitat attributes that are related to habitat occupancy should also influence demographic processes such as recruitment and survival rates (which define habitat quality; Van Horne 1983), and ultimately population extinction and colonisation dynamics (Armstrong 2005). For amphibians, research of this kind has traditionally focussed on relationships between their occurrence in wetlands and the attributes of those wetlands (e.g size, depth, vegetation characteristics, surrounding landscape characteristics; Hazell *et al.* 2001). Significant advances in our understanding of the habitat requirements of amphibians have resulted in information that is now being used to direct wetland conservation and management programs for threatened species (Semlitsch 2000; Marsh and Trenham 2001; Semlitsch 2003; Cushman 2006). However, it is apparent that these programs could also benefit from research on microhabitat use because such studies may identify specific microhabitats, or microhabitats with particular attributes, which individuals rely upon for day-to-day survival or reproductive activities. Nonetheless, microhabitat preferences of threatened amphibians have received limited attention. Amongst anurans, broad microhabitat affiliations have been documented for several threatened taxa in recent years (Gillespie and Hollis 1996; Schley *et al.* 1998; Lemckert and Brassil 2000; Hodgkison and Hero 2001; Lemckert and Slayter 2002; Lemckert and Brassil 2003), but we are aware of only two studies which have utilised statistical techniques to determine whether threatened species utilise particular microhabitats, or microhabitats with particular attributes, preferentially (Griffin and Case 2001; Gillespie 2002).

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[*]Referred to as the Southern Bell Frog in NSW

In this study, we examined microhabitat preferences of the Growling Grass Frog *Litoria raniformis* during nocturnal activity as a preliminary component of research on the habitat requirements of the frog around Melbourne, Victoria, Australia (Robertson *et al.* 2002; Heard *et al.* 2004; G. Heard unpubl. data). This species is listed as endangered in Victoria (DSE 2003), and is listed nationally as vulnerable to extinction under the Commonwealth *Environment Protection and Biodiversity Conservation Act* 1999. We sought to determine whether *L. raniformis* displays preferential microhabitat use during nocturnal activity in two ways: (i) by comparing the use of microhabitat categories to their random availability within wetlands, and; (ii) by comparing the structural attributes of microhabitats used by *L. raniformis* to those of random points within wetlands.

Methods

Study area and sites

The study was conducted within the Merri Creek Corridor (hereafter MCC) on the northern outskirts of the Melbourne metropolitan area (Fig. 1). The MCC comprises a significant proportion of Melbourne's northern basalt plain: a gently undulating, volcanic landscape which rises to a maximum elevation of around 200 m. The climate of the region is temperate with an average annual rainfall of around 600 mm (Beardsell 1997). Soils are characterised by dark basaltic clays interwoven with basalt boulders and outcrops (Anon. 2001). Native terrestrial vegetation consists mostly of grassland, shrubland and River Red Gum *Eucalyptus camaldulensis* woodland (Savio 2001; Beardsell 1997), although non-native pasture is now the most extensive vegetation community. Wetlands include permanent and ephemeral streams, freshwater meadows and marshes, and numerous man-made structures such as farm dams, disused quarries and ponds used for water-treatment or storage. The MCC is undergoing rapid conversion from primarily agricultural land-use to extensive urban and industrial land-use.

Data on microhabitat preferences of *L. raniformis* were collected at six wetland sites within the study area (four lotic, two lentic). These sites were a sub-set of 27 localities monitored in the region during the 2002-2003 summer activity season of the frog (Heard *et al.* 2006). Microhabitat sampling sites were selected based upon prior knowledge of *L. raniformis* abundance, and accessibility and safety considerations. The location of these sites and a summary of their biophysical characteristics are provided in Table 1.

Sampling period and census techniques

Between four and six visual censuses were conducted at each site between January and March 2003 to assess microhabitat use by *L. raniformis*. Whilst this species is nocturnally active from September to April in this study area, activity levels appear to peak in late summer, coinciding with relatively high and stable night-time temperatures (G. Heard pers. obs.). The sampling period was selected to maximise detection rates of nocturnally active frogs. However, this period also coincides with the apparent cessation of reproductive activity by the species in southern Victoria, as deduced by the gradual termination of calling by males and de-pigmentation of nuptial pads in January (G. Heard pers. obs.). The patterns of microhabitat use observed during this study do not necessarily reflect those of the breeding season.

Transects which ran along the water-line were established at each site. Stream transects were generally 50 m in length (Table 1); however, an additional 150 m section of stream between two sites was also surveyed to increase sample sizes of frog microhabitats. Transects traversed the entire circumference of lentic sites. Transects incorporated the aquatic zone and the riparian zone to a distance of 15 m perpendicular to the water-line. *Litoria raniformis* are most frequently detected within this zone during spotlight surveys at wetlands within the study area (G. Heard, P. Robertson pers. obs.).

Census techniques are described elsewhere (Heard *et al.* 2006). Briefly, visual census of transects was undertaken with the aid of spotlights between 2100 and 0300 hours by two people following procedures outlined by Crump and Scott (1994). Individual *L. raniformis* were detected by direct encounter or by eye-shine. We attempted to minimize bias in the detectability of frogs between microhabitats by carefully inspecting structurally complex microhabitats where the visibility of frogs is likely to have been lower. To standardise detection rates, surveys were only undertaken when weather conditions were considered favourable for nocturnal activity by *L. raniformis* (see Heard *et al.* 2006). Furthermore, the sequence of surveys and the direction of travel along each transect were randomised to minimise bias in frog activity between and within sites. Measures to reduce the risk of spreading infectious pathogens (such as chytrid fungus) between the survey sites were implemented (NPWS 2001).

Microhabitat sampling

Prior to this study, only anecdotal information was available on the microhabitat use of *L. raniformis* (Pyke 2002). The categorisation of microhabitats and selection of structural attributes for microhabitat sampling was subsequently guided by our knowledge of the species' habitat and microhabitat affiliations within the study area (Robertson *et al.* 2002), and that were used in comparable studies (McAlpine and Dilworth 1989; Gillespie *et al.* 2004).

Upon detecting individual *L. raniformis*, we noted whether the frog was located in the riparian or aquatic zone of the wetland, and recorded the microhabitat occupied as either: (i) on bare soil; (ii) on bank-side rock; (iii) on leaf litter; (iv) on or in ground vegetation; (v) on or in emergent vegetation; (vi) in fringing vegetation; (vii) on or in floating or submergent vegetation; (viii) on emergent rock, or; (ix) in open water. The exact location of each frog was marked using flagging tape or small fluorescent floats, and the structural attributes of each microhabitat measured the following day.

Structural attributes of microhabitats were measured using a 50 cm square quadrat. Definitions of these attributes are provided in Table 2. The quadrat was constructed of 35 mm diameter PVC pipe, divided into 25 identical squares using five evenly spaced vertical and horizontal string lines (McAlpine and Dilworth 1989). To measure structural attributes, the central grid-square of the quadrat was positioned on the exact location at which the frog

Table 1. Details of the six sampling sites at which data on the microhabitat preferences of the Growling Grass Frog *Litoria raniformis* were collected between January and March 2003.

Site reference number	**Location**		**Wetland type**	**Wetland sub-type**	**General biophysical characteristics**	**Live-stock grazing frequency**	**Transect length (m)**	**GGF microhabitats examined**
	Latitude	Longitude						
Donnybrook								
D003	37°32'26"	144°58'11"	Lentic	Farm dam	Large dam with no over-storey and very little under-storey vegetation, sparse pasture on earthen banks with little rock and patches of emergent vegetation, very sparse emergent and submergent vegetation in aquatic zone, maximum water depth of 3 m	Constant	254	24
D019	37°32'35"	144°57'31"	Lotic	Semi-permanent stream	Shallow drainage line with sparse over- and under-storey vegetation, sparse pasture on earthen banks with occasional basalt boulders and patches of emergent vegetation, patchy emergent, submergent and floating vegetation in the aquatic zone, still to slow flowing water, maximum water depth of 1.2 m	Constant	50	32
Somerton								
S004-S005	37°37'56"	144°57'24"	Lotic	Permanent stream	Incised gorge environment with little over-storey but often dense under-storey vegetation, mixture of native and introduced grasses on earthen banks with numerous basalt boulders and patchy emergent vegetation, mosaic of emergent, submergent and floating vegetation in aquatic zone, slow to rapid flowing water, maximum water depth of 1 m	Occasional	250	39
S008	37°37'27"	144°57'07"	Lotic	Permanent stream	As for S004-S005	Occasional	50	13
S018	37°38'18"	144°57'53"	Lotic	Permanent stream	As for S004-S005	Occasional	50	14
Wollert								
WL001	37°35'39"	145°02'42"	Lentic	Water-storage pond	Large water-body used to store water within a quarry property, no over-storey and only sparse under-storey vegetation, sparse pasture on earthen banks with occasional basalt boulders and patches of emergent vegetation, sparse emergent and submergent vegetation in aquatic zone, maximum water depth of 1.5 m	Constant	516	28

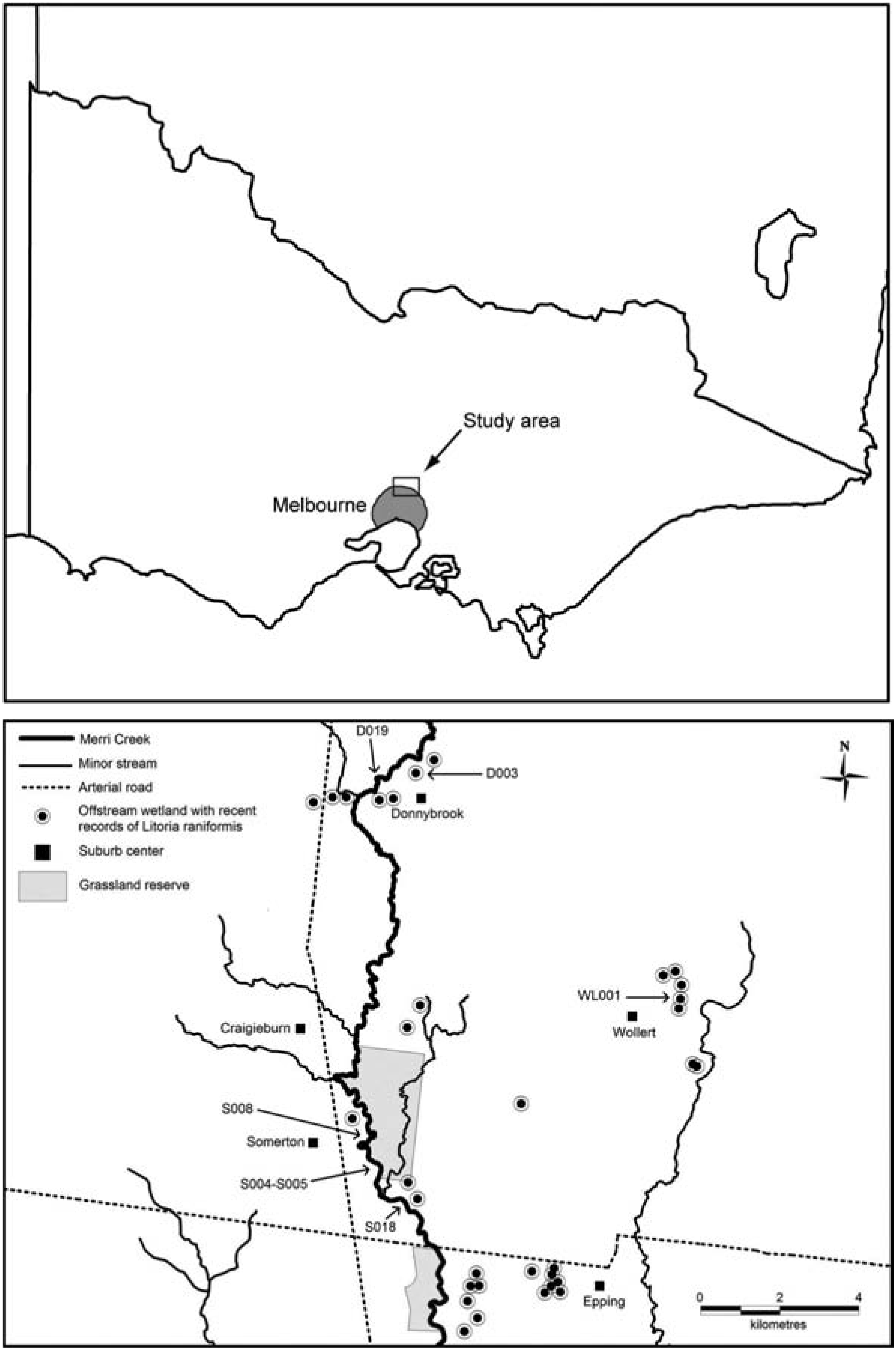

Study area
Melbourne
Merri Creek
Minor stream
Arterial road
Offstream wetland with recent records of Litoria raniformis
Suburb center
Grassland reserve
D019
D003
Donnybrook
N
WL001
Wollert
Craigieburn
S008
Somerton
S004-S005
S018
Epping
0
2
4
kilometres

Figure 1. Map of the study area, showing its location within Victoria (top panel) and the spatial relationship between study sites (bottom panel).

was recorded the previous night. For microhabitats in the riparian zone, the slope of the bank was measured in degrees using a clinometer (Model PM-5/360PC, Suunto Co., Helsinki, Finland). For aquatic microhabitats, the maximum water depth within the quadrat was measured using a pole marked at 10 cm intervals. Distance to the water-line was also measured in both zones with this pole. All remaining attributes were scored visually. For microhabitats in the riparian zone, characteristics of the substrate were quantified by counting the number of grid cells in which each substrate type predominated. For aquatic microhabitats, only the predominant substrate was recorded due to difficulties in viewing the substrate through the water. The number of grid-cells containing rocks that emerged above the water's surface was counted, and water-flow rate scored as still, slow or rapid. Vegetation attributes were restricted to the dominant macrophytic forms. However, algae that grew in continuous mats on the water surface were included within the 'floating vegetation' category (see Table 2) because of previous observations of *L. raniformis* perching upon it (G. Heard, P. Robertson pers. obs.). The diversity of plants within each vegetation category was recorded, as were several measures of foliage cover. For consistency, the senior author collected all data.

Table 2. Definitions of structural attributes measured for microhabitats of the Growling Grass Frog *Litoria raniformis* and random points within each study site.

Attribute	Riparian or aquatic zone measurement	Measure
Physiography		
DISTWATER	Riparian	Horizontal distance to the water-line (+/- 10 cm)
SLOPE	Riparian	Slope of bank in degrees
BAREROCK	Riparian	Number of grid squares with substrate predominantly bare rock
BARESOIL	Riparian	Number of grid squares with substrate predominantly bare soil
LITTER	Riparian	Number of grid squares with substrate predominantly leaf litter
DISTBANK	Aquatic	Horizontal distance to the water-line (+/- 10 cm)
SUBSTRATE	Aquatic	Predominant substrate categorized as either bare-rock (continuous sheets of rock), rock rubble (fractured rock), gravel (grains >1 mm^3), sand (grains <1 mm^3) or mud (fine silt).
EMERGENTROCK	Aquatic	Number of grid squares containing rocks which emerged above the water's surface
WATERFLOW	Aquatic	Flow of the water as either still (no flow), slow (flow <1 m/sec) or rapid (flow >1 m/sec).
WATERDEPTH	Aquatic	Maximum depth of water in plot (+/- 10 cm)
Vegetation		
1. Measurements		
DIVERSITY	-	Count of all species in plot
MINIMUM	-	Visual estimate of the lowest foliage cover in any grid-cell in plot
MAXIMUM	-	Visual estimate of the highest foliage cover in any grid-cell in plot
RANGE	-	Maximum minus the minimum cover in plot
MEAN	-	Visual estimate of the mean foliage cover in plot
EXTENT	-	Count of grid-cells with some foliage cover in plot
WOODYSTEMS	-	Count of woody stems rooted in plot
2. Types		
FRINGINGVEG	Both	All woody under-storey and over-storey vegetation
SPARSEGROUNDVEG	Riparian	All grasses, herbs, forbs and non-woody weeds <30 cm tall, not forming a continuous cover of the substrate
DENSEGROUNDVEG	Riparian	All grasses, herbs, forbs and non-woody weeds >30 cm tall forming a continuous cover of the substrate
EMERGENTVEG	Both	All plants with a growth form specialized to be rooted below the water with foliage emerging above the water
FLOATINGVEG	Aquatic	All plants with a growth form specialized to have foliage that floats on the water surface, including rooted or free-floating species
SUBMERGENTVEG	Aquatic	All plants with a growth form specialized to be rooted below the water with foliage below the water surface

To determine whether *L. raniformis* displayed preferential microhabitat use at these sites, we measured the same attributes recorded for frog microhabitats at random points within the riparian and aquatic zone. The number of random points sampled in each zone matched the number of frog microhabitats sampled. Random points were located using random combinations of x and y co-ordinates, where x was a horizontal distance perpendicular to the water-line and y was a horizontal distance along the transect. To measure microhabitat attributes, the quadrat was placed with the central grid over the exact location of the random site. The most prominent structural attribute of the 10 cm square area of the central grid square (e.g. bare-soil, emergent vegetation etc) was used to assign each random site into one of the nine microhabitat categories described above. Measurement of structural attributes of random sites followed the same procedures used for frog microhabitats.

Data analysis

Due to biophysical differences between the lotic and lentic study sites, and the riparian and aquatic zones within them, data were separated according to wetland type and zone prior to analysis. However, as frogs were rarely encountered in the aquatic zone of lentic sites, these data were excluded from further analyses. Frog observations were treated as independent samples throughout the analysis. While this has the potential to introduce bias if individual frogs contributed multiple samples, we consider this to be unlikely based upon low recapture rates of marked individuals recently obtained at these sites using survey techniques identical to those used here (G. Heard unpubl. data).

Chi-square goodness of fit tests were used to determine if the use of microhabitats by *L. raniformis* differed from their random availability. Due to small sample sizes, data were pooled across sites within each wetland type and zone for this analysis. As some microhabitats still displayed expected values less than five, these samples were combined with structurally similar microhabitats prior to testing.

Non-metric multidimensional scaling (NMDS; Kruskal 1964) was employed to examine differences in the structural attributes of *L. raniformis* microhabitats and those of random points, following Hollis (2004) and Gillespie *et al.* (2004). Data screening was first undertaken to remove superfluous variables. Due to limited variation in the substrate of aquatic frog microhabitats and random points, this attribute was excluded. Co-linearity between remaining variables was examined using a Spearman's rank correlation (r_s) matrix. The measures of vegetation attributes were often highly correlated in each data-set ($r_s > 0.80$). As the extent of vegetation cover represents an objective measurement, it was selected for inclusion in all subsequent analyses. Structural data were range-standardized, such that the highest value for each attribute was equal to one, and the lowest to zero. A matrix of pair-wise dissimilarities between frog microhabitats and random points was constructed based upon the structural data. Euclidean distance was used as the dissimilarity metric. Ordination of the dissimilarity matrix was undertaken using NMDS. This procedure constructs ordination plots in which there is maximal correlation between the rank-order of distance between points in a pre-arranged number of spatial dimensions and the rank-order of similarity between points in the original dissimilarity matrix. Agreement between the inter-point distances and the dissimilarity matrix is measured by a stress function with possible values between 0 and 1, where 0 indicates perfect agreement between the rank-orders. To enable interpretation of the relationships between the dimensions of the ordination space and the original structural attributes, linear vectors were fitted to the ordination diagram. These vectors allow the distribution of frog microhabitats and random points within the plots to be visually related to variation in the original structural attributes. The statistical significance of differences in the structural attributes of frog microhabitats and random points was tested using analysis of similarities (ANOSIM; Clarke 1993). ANOSIM tests were based upon 10000 random permutations of the dissimilarity matrix, with permutations stratified within sites. All analyses were undertaken with the R statistical software package, version 2.4.1 (R Development Core Team 2006). Fitting of vectors and ANOSIM procedures were undertaken with the R add-in package *vegan* (Oksanen *et al.* 2007), following the methodology originally proposed by Minchin (1991).

Results

Microhabitat use

Use of microhabitat categories by *L. raniformis* (Fig. 2) differed significantly to their observed availability in the riparian and aquatic zones of both the lotic and lentic study sites (Lotic sites: riparian zone, $\chi^2 = 85.60$, d.f. = 2, $P < 0.001$; aquatic zone, $\chi^2 = 18.80$, d.f. = 2, $P < 0.001$. Lentic sites: riparian zone, $\chi^2 = 12.29$, d.f. = 1, $P < 0.001$). Trends in microhabitat use in the riparian zone were similar in both wetland types. Frogs were most frequently observed perched on bare soil and bank-side rocks, and at rates exceeding the availability of these microhabitats (Fig. 3). Frogs were infrequently observed on leaf litter or in emergent or fringing vegetation, but this conformed to the availability of these microhabitats. Most striking was the species' apparent avoidance of ground vegetation (Fig. 3). Whilst some individuals were located amongst sparse, grazed pasture grasses (vegetation height < 10 cm), observations of frogs perched in dense ground vegetation were very rare.

In the aquatic zone of lotic sites, 60% of *L. raniformis* were observed on or in dense mats of floating and submerged vegetation. This greatly exceeded the availability of this microhabitat type (Fig. 3). Both adults and juveniles were observed utilising these mats, which were composed of either free-floating species such as *Azolla pinnata*, *Lemna* sp. and algae, or the foliage of plants which were rooted in the substrate including *Triglochin procerum*, *Potamogeton crispus*, *P. pectinatus* and *P. tricarinatus*. Frogs were also frequently observed perched in emergent vegetation (mainly *Eleocharis sphacelata*, *Rumex bidens*, *Schoenoplectus validus* and *Typha* spp.); however, only at a rate that marginally exceeded its availability. Few frogs were located in fringing vegetation or on emergent rocks (reflecting their relative scarcity) and none were located in open water.

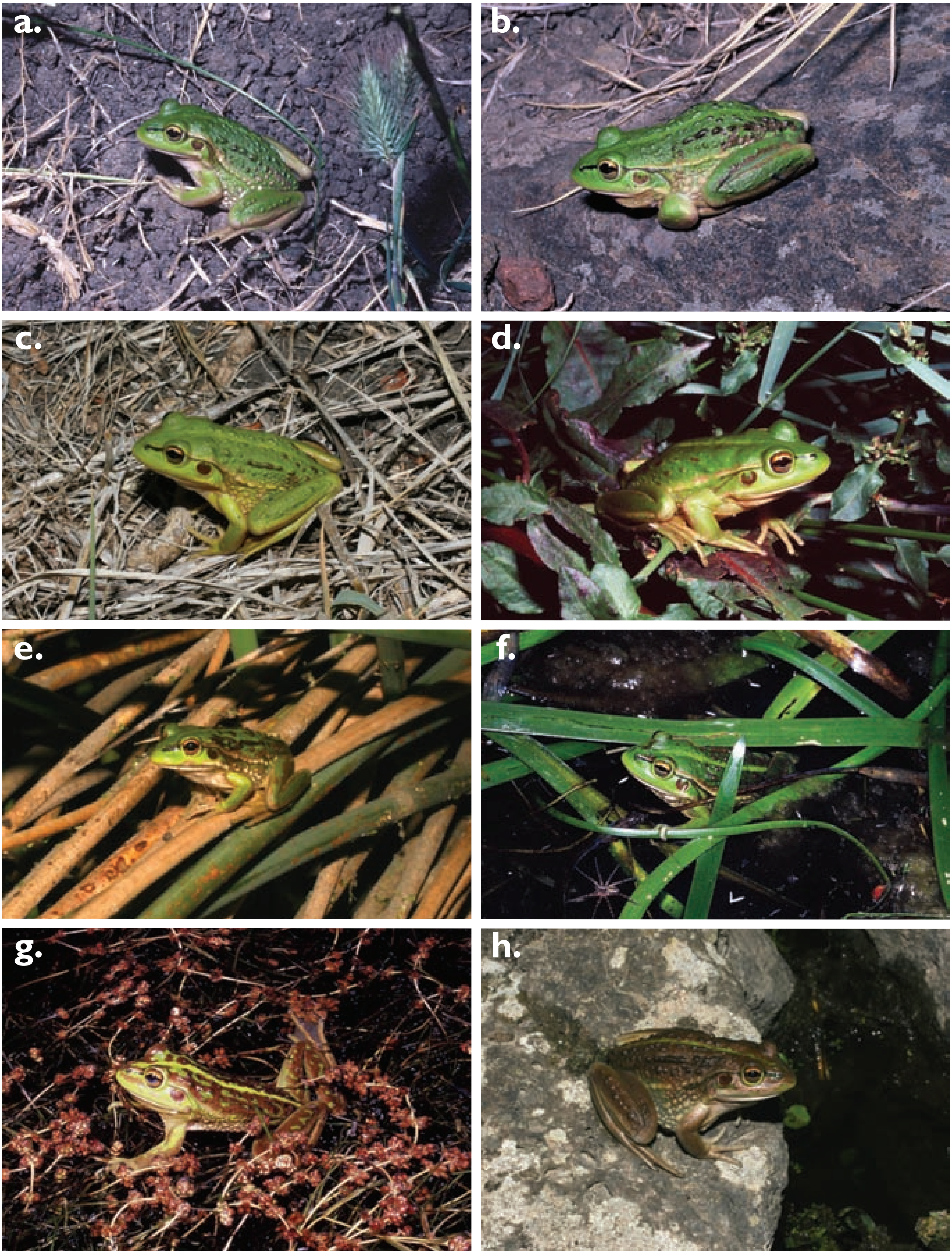


Figure 2. *In situ* photographs of nocturnal microhabitat use by Growling Grass Frogs *Litoria raniformis* within the study area. Microhabitats include (a) bare soil; (b) bank-side rock; (c) leaf litter; (d) ground vegetation; (e) emergent vegetation (*Schoenoplectus validus*); (f) floating vegetation (*Triglochin procerum*); (g) submergent vegetation (*Potamogeton pectinatus*); (h) emergent rock.

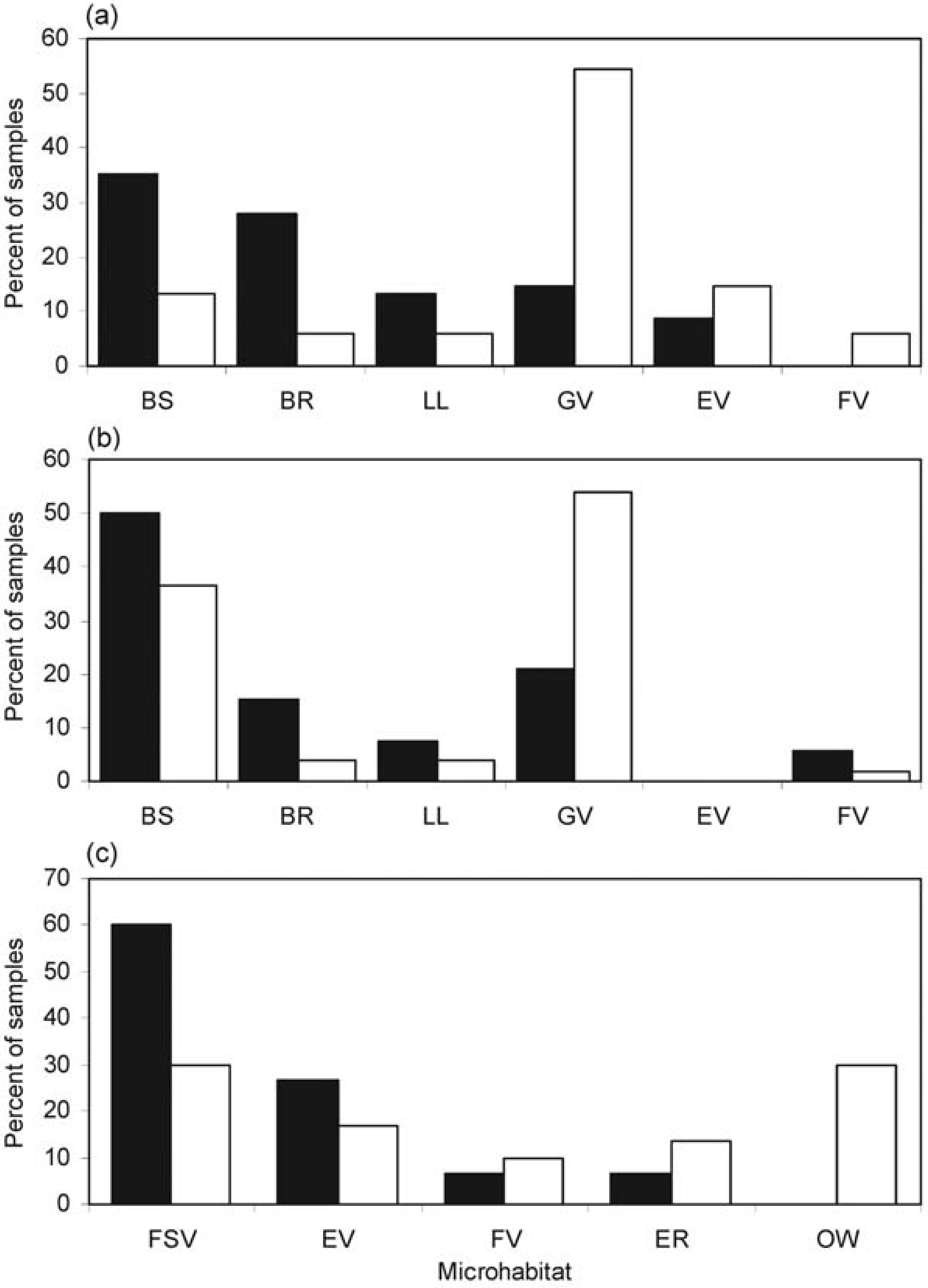
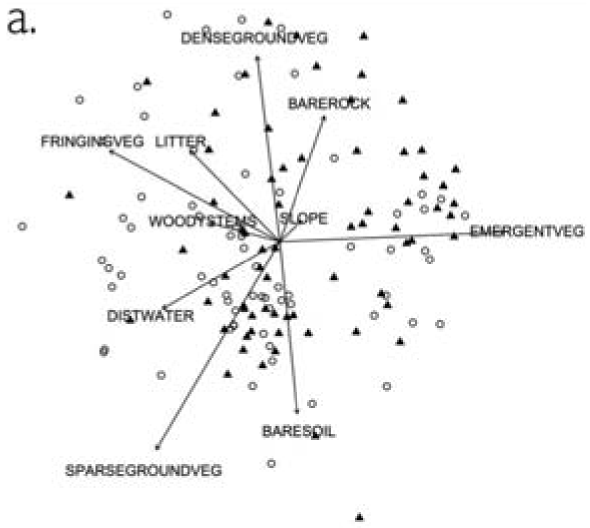


Figure 3. Microhabitat use of the Growling Grass Frog *Litoria raniformis* compared to the random availability of those microhabitats within (a) the riparian zone of lotic sites, (b) the riparian zone of lentic sites, and (c) the aquatic zone of lotic sites. Microhabitat use by *L. raniformis* (filled bars) is defined as the percent of observations of frogs within each of the different microhabitat categories, whereas microhabitat availability (open bars) is based upon the percent of random points that were assigned to each microhabitat category. Data were pooled across sites. Microhabitats are: BS, bare soil; BR, bank-side rock; LL, leaf litter; GV, ground vegetation; EV, emergent vegetation; FV, fringing vegetation; FSV, floating and submergent vegetation; ER, emergent rock; OW, open water.

Structural attributes of microhabitats

Ordination revealed differences in the structural attributes of *L. raniformis* microhabitats and random points in each wetland type and zone (Fig. 4). Stress values were relatively low for each ordination (riparian zone of lotic sites, stress = 0.23; riparian zone of lentic sites, stress = 0.15; aquatic zone of lotic sites, stress = 0.14) indicating good concordance between the dissimilarity matrices and the ordination configurations. Differences in the structural attributes of frog microhabitats and random points were statistically significant for each data-set (ANOSIM: riparian zone of lotic sites, $R = 0.04$, $P = 0.002$; riparian zone of lentic sites, $R = 0.12$, $P < 0.001$; aquatic zone of lotic sites, $R = 0.23$, $P < 0.001$).

In the riparian zone of lotic sites, microhabitats of *L. raniformis* were structurally variable (Fig. 4a). However, examination of vectors indicates that they did not display the high cover of fringing vegetation, leaf litter, woody stems and dense ground vegetation, and low cover of emergent vegetation, of some random points. They also generally occurred in areas closer to the water-line. Numerous frog microhabitats in the riparian zone of lotic sites displayed relatively high bare soil and sparse ground vegetation cover, but many random points did also. Microhabitats of *L. raniformis* were more clearly separated from random points in the riparian zone of lentic sites (Fig. 4b). Here, the majority of frog microhabitats were characterised by a relatively high cover of bare soil and bare rock, low cover of emergent vegetation, steeply-sloping bank and close proximity to the water-line, whereas the opposite was true for random points. Microhabitats of *L. raniformis* were also clearly separated from random points in the aquatic zone of lotic sites based upon their structural attributes (fig. 4c). Vectors indicate that this separation results from frog microhabitats displaying greater floating vegetation cover and water depth, and lower water flow rates and emergent rock cover.



Discussion

This study demonstrates that *Litoria raniformis* displays preferential use of certain microhabitats during nocturnal activity in wetlands on the northern outskirts of Melbourne, Victoria. Nonetheless, we have assumed that the detectability of frogs occupying microhabitats with differing structural characteristics was equal. If this was not the case, observed microhabitat preferences would be influenced by detection rates. We cannot discount this possibility. However, attempts were made to minimise any such bias by carefully inspecting structurally complex microhabitats where the visibility of frogs is likely to have been lower. These additional searches rarely revealed frogs that were not seen initially. We are confident that the results of this study are not greatly affected by heterogeneity in detection rates.

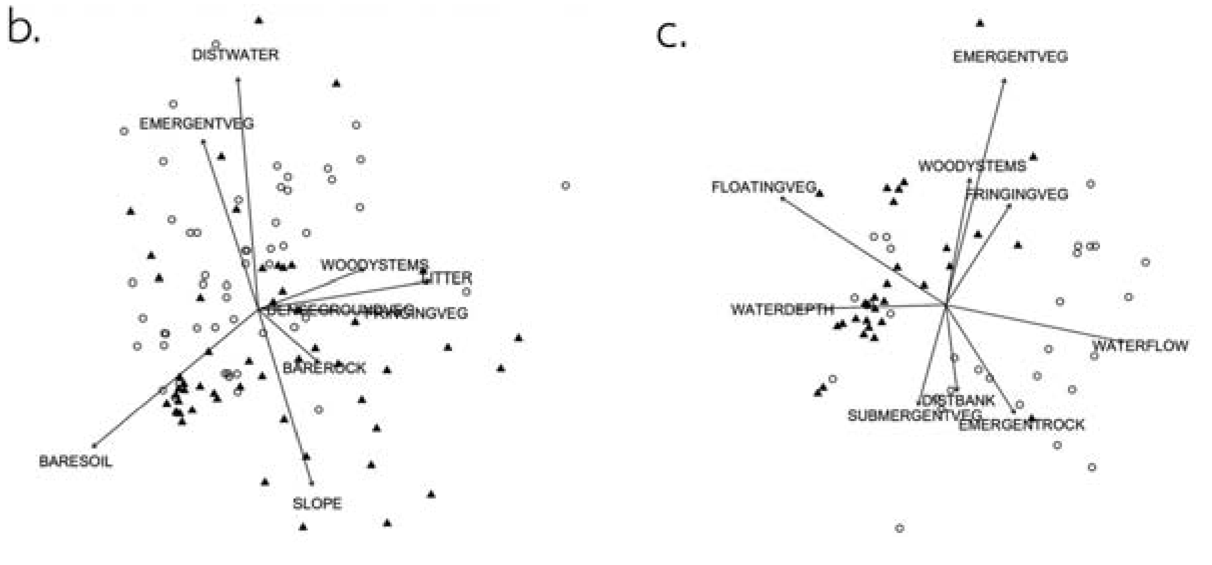


Figure 4. Plots showing the distribution of microhabitats of the Growling Grass Frog *Litoria raniformis* and random points within 2-dimensional NMDS ordinations based upon their structural attributes. Plots represent these distributions within (a) the riparian zone of lotic sites, (b) the riparian zone of lentic sites, and (c) the aquatic zone of lotic sites. (▲), frog microhabitats; (○), random points. The fitted vectors superimposed on each plot represent the directional relationship between the ordination space and the measured structural attributes. The strength of the relationships is proportional to the length of the vectors. See Table 2 for attribute definitions.

In both lotic and lentic sampling sites, the majority of frogs located in the riparian zone were perched on bare soil, rocks or leaf litter close to the water's edge, and few were located in stands of ground vegetation relative to the high availability of this microhabitat. Considering that few frogs were located in emergent or fringing vegetation, these results indicate a preference for terrestrial microhabitats with relatively low structural complexity in the vertical plane. The structural data collected at lentic sites support this contention: microhabitats occupied by *L. raniformis* were consistently located on steeply-sloping sections of bank, close to the water line with high bare soil and bare rock cover, and low emergent vegetation cover. Structural data collected in the riparian zone of lotic sites do not show such a clear pattern but still tend to support the observation that microhabitats of *L. raniformis* displayed relatively low structural complexity in the vertical plane. Unlike many random points, few frog microhabitats displayed a high cover of fringing or dense ground vegetation, or a high density of woody stems. Many frog microhabitats displayed relatively high emergent vegetation cover, which may appear contrary to this trend. However, differences in the emergent vegetation between lotic and lentic sites explain this apparent discrepancy. At the lentic sites, emergent vegetation consisted entirely of *Eleocharis acuta* and the weed *Juncus acutus* that generally grew as dense patches of vertical foliage. This contrasts with the taller, less rigid emergent vegetation that predominated at lotic sites (*Schoenoplectus validus*, *Typha* spp., *Eleocharis sphacelata*) which often grew in clumps separated by rocks or areas of bare soil exposed by cattle grazing. Most frogs were located on these structures rather than in the emergent vegetation itself, and when perched in emergent vegetation were generally on horizontal rather than vertical foliage.

The preference of *L. raniformis* for microhabitats with low vertical structural complexity was also evident in the aquatic zone. The great majority of frogs were located in or on mats of floating and submergent vegetation. Emergent vegetation was also frequently utilised but frogs were generally perched on the platforms of horizontal stems referred to above. Analysis of structural data showed that occupied microhabitats were more frequently characterised by high floating vegetation cover over still, deep water than by high emergent or fringing vegetation cover, or high woody stem density.

There are several possible explanations for the microhabitat preferences documented in this study. *Litoria raniformis* is known to forage nocturnally, pursuing prey including a variety of terrestrial and aquatic invertebrates, and small vertebrates (Pyke 2002; G. Marentelli pers. comm.; G. Heard, P. Robertson, M. Scroggie pers. obs.). Like the closely-related Green and Golden Bell Frog (*Litoria aurea*), *L. raniformis* employs an 'ambush' or 'sit-and-wait' foraging mode to capture such prey (Christy 2001; Miehs and Pyke 2001; G. Heard pers. obs.). The selection of microhabitats during nocturnal activity that have little surrounding vertical structure would aid the frog's ability to see and capture prey in both terrestrial and aquatic situations. Tactics for thermoregulation, hydroregulation and predator avoidance may also have influenced the microhabitat preferences documented here. Thermoregulation and hydroregulation both place major physiological constraints on the activity of amphibians (Duellman and Trueb 1986), and should therefore influence microhabitat selection. In both cases, this influence is likely to have primarily manifested in the choice between a terrestrial or aquatic microhabitat for frogs sampled in this study because variation in microhabitat temperature and humidity (or other external factors related to hydroregulation) within zones is likely to have been much lower than between zones. Nonetheless, the preference for microhabitats closer to the water-line in the riparian zone may have been influenced by these factors. During conditions when thermoregulatory and hydroregulatory stress are negligible (such as warm nights with light rain or fog), individual *L. raniformis* can occasionally be found in apparent foraging positions up to 100 m from the water-line of wetlands in the study area (G. Heard pers. obs.). It is also plausible that microhabitats close to the water-line are superior for avoiding predators during activity in the riparian zone because this species' main tactic in this regard is to retreat to the water (as deduced by their response to approaching humans; G. Heard, P. Robertson, M. Scroggie pers. obs.). Experiments are ultimately required to determine how these factors affect microhabitat selection by *L. raniformis* during its nocturnal activity period.

Several recent studies have examined relationships between wetland attributes and wetland occupancy by *L. raniformis* across Melbourne's urban-fringe (Robertson *et al.* 2002; Heard *et al.* 2004; Poole 2004; Hamer and Organ 2008). As wildlife species are thought to occupy habitats that increase their ability to survive and reproduce (Fretwell and Lucas 1970), attributes related to wetland occupancy may also influence habitat quality for these frogs in this region. Microhabitat needs are of interest in this regard as they represent a potential mechanism through which such influence is mediated. Each of these studies demonstrates a positive relationship between the cover of floating and/or submergent vegetation and wetland occupancy by *L. raniformis*. Our observation that dense mats of floating and submergent vegetation are an important resource for adult frogs provides some support for a positive relationship between this attribute and habitat quality. Two of the above studies also demonstrated a positive relationship between emergent vegetation cover and wetland occupancy by *L. raniformis*. Structurally diverse patches of emergent vegetation were utilised by these frogs during nocturnal activity in our study sites, and preferentially so in the aquatic zone. They were also utilised as basking sites during diurnal activity (G. Heard pers. obs.), as has been observed elsewhere (Pyke 2002). Nonetheless, nocturnally active frogs sampled in this study generally avoided dense emergent vegetation, and there was a preference for microhabitats that had relatively low structural complexity in the vertical plane. If emergent vegetation cover is related to habitat quality for *L. raniformis*, there may be an optimum coverage above and below which habitat quality decreases.

The remaining habitat attributes considered in this study appear unrelated to wetland occupancy by *L. raniformis* in the landscapes of outer Melbourne (Robertson *et al.* 2002; Heard *et al.* 2004; Poole 2004; Hamer and Organ 2008). From this study, the preference of these frogs for microhabitats such as bare soil and bare rock in the riparian zone, and avoidance of dense ground vegetation, suggests that areas of the bank with little vegetation cover may be an important resource.

This and other recent studies have contributed insights into the habitat requirements of *L. raniformis* in Melbourne's urban-fringe environments, and provide guidance to further research and conservation planning. In terms of microhabitat preferences, knowledge of those displayed during other times of year (e.g. breeding season, over-wintering period) or in other activity states (e.g. sheltering, calling etc.) would be useful. However, research that seeks to quantify relationships between wetland attributes and demographic processes is critical, because an understanding of these relationships is required to predict the consequences of future habitat changes. In the interim, habitat management plans for *L. raniformis* in this region should incorporate preservation of floating, submergent and emergent vegetation, but also recognise the possible requirement of this species for areas with minimal structural complexity. Given this point, and evidence that the demographic processes of *L. raniformis* (like other amphibians) are affected by broader landscape processes (Robertson *et al.* 2002; Heard *et al.* 2004; Poole 2004; Hamer and Organ 2008), management plans should incorporate protection of networks of wetlands in which habitat conditions vary spatially and temporally.

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